Sci. J. Fac. Sci. Menoufia Univ.
vol. III (Botany & Zoology)1989.

HISTOLOGICAL EFFECTS OF IRRADIATION ON THE GONADS OF SPODOPTERA LITTORALIS (Boisd).

BY

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ABSTRACT

The effect of ionizing of gamma irradiation on the testes and ovaries of adults of Spodoptera littoralis emerged from 1, 3 and 5-day- old pupae, using doses of 50, 100, 150, 200 and 300 Gy, were studied. The irradiation caused a significant bad effect on both testes and ovaries. The effect was more pronounced by increasing the dose. The high dose was found to be most effective for producing sterility specially when the five days old pupae of S. littoralis were irradiated with 300 Gy dose. It is also worthy mentioned that mature cells were more resistant than transitional cells.

INTRODUCTION

The modifications that ionizing radiations induce (histological change) on the gonads have been studied with

the beginning of radiobiological applied reaserch.

Few years ago many workers reported that the females of insects were more sensitive to gamma irradiation than the males. The female adults of Tenebrio malitor were sterilized by a dose of 5 Krad, whereas the male adults required a dose 3 times as high before sterility occurs, Brower (1973) and Ashref and Brower (1974). Baccetti and De Dominicis (1964) on Dacus oleae; El-Halfawy (1983) on Spodoptera littoralis, Srivastava et al (1985) on Dysdereus koenigii and Rosada (1988) on Anagasta kuehniella. They reported that spermatids and sperm showed high resistance to gamma radiation than spermatogonia and spermatocytes. Little is known about the effect of ionizing radiation on S. littoralis.

Therefore the aim of this study is to elucidate the histological changes in the gonads of adult cotton leaf worm Spodoptera littoralis exposed to different doses of gamma radiation.

MATERIAL AND METHODS

The strain of cotton leaf worm, Spodoptera littoralis, used for this research were brought from Faculty of Science, Menoufia University, and reared under laboratory condition of 25±2°C and 70±5% R.H. Pupae were collected

and segregated by sex. A total of 120 pupae aged one, three and five days old were irradiated with CO^{60} at a rate of 1000 r/min. Doses of 50, 100, 150, 200 and 300 Gy of gamma radiation were used.

For histological study, the emerged adults were dissected in saline solution. Testes and ovaries were uprooted and fixed for 12 hr in alcoholic Bouin's fixtive.

Gonads were then dehydrated in ascending alcoholic series and cleared in xylol before embedding in paraffin wax 55:57°C M.P. in order to prepare them for sectioning. The sections were cut 7 microns thick and stained with Delafields haematoxylin and Eosin stain.

RESULTS

Normal structure of S.littoralis testes:

Each testis is formed of 8 follicles. Each follicle contains a successive zones in which germ cells are located. At the distal end of each follicular chamber, there is a large nucleated mass of protoplasm known as apical cells. Within each follicle there is a distinct germinal area consisting of spermatogonia. These cells are rounded in shape, containing large nuclei. Spermatogonia are grouped in the form of cyst in which they are transformed to spermatocytes. All cells within the cyst

mature to spermatid (Immature sperm). These cells are in stage of synchronous transformation into spermatozoa (mature sperm). Sperms are nearly spherical (Fig. 1).

Treated testes:

The testes of adults which emerged from one day old pupae irradiated with 50 Gy, showing all stages of spermatogenesis. The germinal area appeared normal but the testicular follicular tissue was destructed. The follicular septum was completely absent. Although no difference were observed at 50 Gy dose, the adults emerging from irradiated one day old pupae treated with 100 Gy, showed that the germ cells in most stages were present but their number was less than untreated testis which means that the formation of spermatids from spermatocytes had been stopped. Spermatids were seen enclosed in a broken cyst. Immature sperm bundles appeared taking different morphological shapes being avoid or twisted. Most of the follicular septum was thickened showing extansive vacuolization. Some of them appeared normal in shape (Fig. 2 a,b).

By increasing the dose, the effects were pronounced in adults emerged from irradiated one day old pupa treated with 150 Gy. Many effects were noted for example, damaged germinal area, with deeply stained chromatin material of their nuclei. Spermatocytes were enlarged, spermatid bundles and sperms appeared abnormal (Fig. 3).

Continued

Species	North Egypt		South Egypt	
	El Arish No.	Sinai No.	Luxor No.	El Khargah No.
Jassidae				
Chiasmus conspurcatus per.	4	-	1	-
Lygidae				
Oxycarnus hyalinipenus	-	-	3	-
Miridae				
Cyrtopeltis tenuis Reut.	1	1	1	-
Nasocoris albipennis Linn.	-	2	1	-
Pentatomidae				
Brachynema cinctum F.	1	-	-	-
Eurydema ornatum L.	3	5	-	2
Eusarcoris inconspicus H.S.	2	1	8	-
Nezera viridula	10	2	5	-
Neuroptera				
Myrmeleontidae				
Creoleon africanus Ramb.	-	3	7	1
Creoleon klugi Navas	2	-	-	-
Creoleon indigus Navas	1	2	5	-
Lepidoptera				
Nymphalidae				
Vanessa atalanta L.	2	10	-	1
Vanessa cardui L.	7	9	5	-

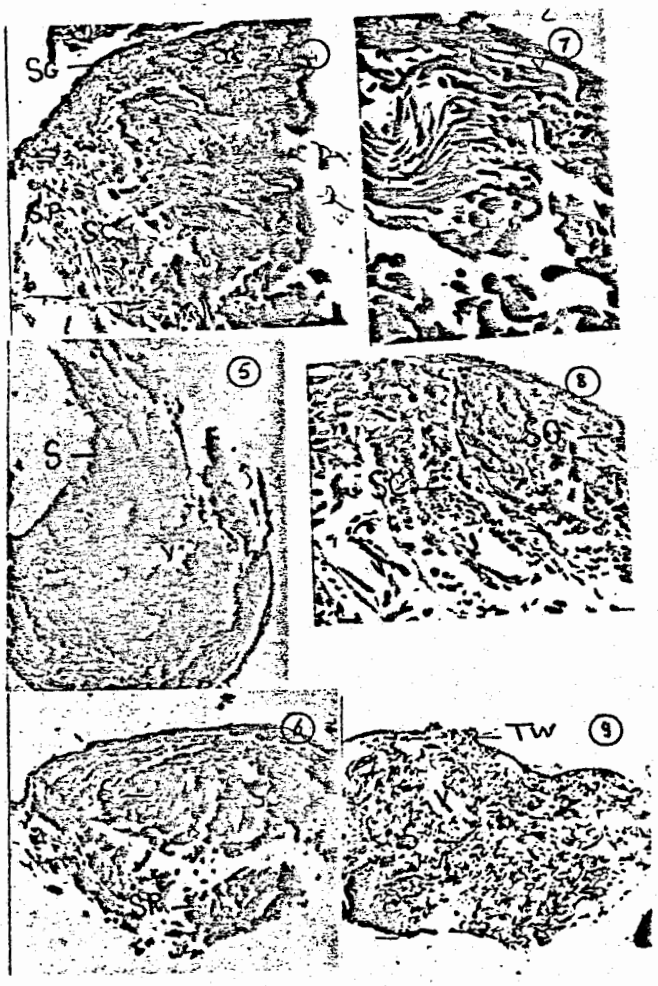
Continued

Species	North Egypt		South Egypt	
	El Arish No.	Sinai No.	Luxor No.	El Khargah No.
Pieridae				
Colias crocea Geoffery	6	10	-	3
Colotis fausta oliv.	-	8	1	2
Pieris rapae L.	20	12	6	-
Pontia glauconma Klug.	3	3	5	-
Sphingidae				
Hippotion celerio L.	3	12	5	-
Hyles liveroneca Esp.	-	6	-	-
Diptera				
Asilidae				
Apoclea algira L.	-	3	-	1
Promachus argentipennis Effl.	-	1	-	-
Bombyliidae				
Anthrax tripunctata Wied.	-	1	-	-
Exoprosopa decrepita Wied.	-	1	-	3
Calliphoridae				
Chrysomia albiceps Wied.	2	6	2	1
Lucilia sericata Mg.	2	2	1	-
Conopidae				
Conops elegans Mg.	-	2	-	1
Myopa dorsalis F.	-	-	-	2

present in some cases. Spermatocytes were degenerated and often lacking cytoplasm, leaving almost empty cysts. In some follicles the spermatocytes were completely lost. Spermatids and sperms were abnormal and appeared necrotic. Immature sperms were seen scattered here and there and mature spermatozoa were degenerated indicating that a dose of 300 Gy is sufficient to arrest the process of spermatogenesis (Fig. 6).

The testes from adults emerged from treated five days old pupae with 100 Gy exhibited all stages of spermatogenesis. The germinal area was normal in appearance. Few changes appear such as vacuoles under testicular follicle (Fig. 7).

Although no differences were observed in sections of testes from adults after treatment with 100 Gy, the sections of adult testes emerging from irradiated five days old pupae with 200 Gy revealed relatively significant damage. Spermatogonia showed a destruction, cyst membranes of spermatocytes were often broken. Spermatids, sperms and testicular wall appear more or less normal (Fig. 8). The effects were more pronounced with the increase in dose. A severe destruction occur in the gonads of adults emerged from treated five days old pupae at dose 300 Gy. The effects seen were in the form of thickened and destructed testicular wall. Abnormal germ cells were observed in



Sections in tests of S. Littoralis:

4) Testis of adult emerged from irradiated 3-day-old pupae with 100 Gy.
5) Testis of adult emerged from irradiated 3-day-old pupae with 200 Gy.
6) Testis of adult emerged from irradiated 5-day-old pupae with 300 Gy.
7) Testis of adult emerged from irradiated 5-day old pupae with 100 Gy.
8) Testis of adult emerged from irradiated 5-day-old pupae with 200 Gy.
9) Testis of adult emerged from irradiated 5-day-old pupae with 300 Gy.

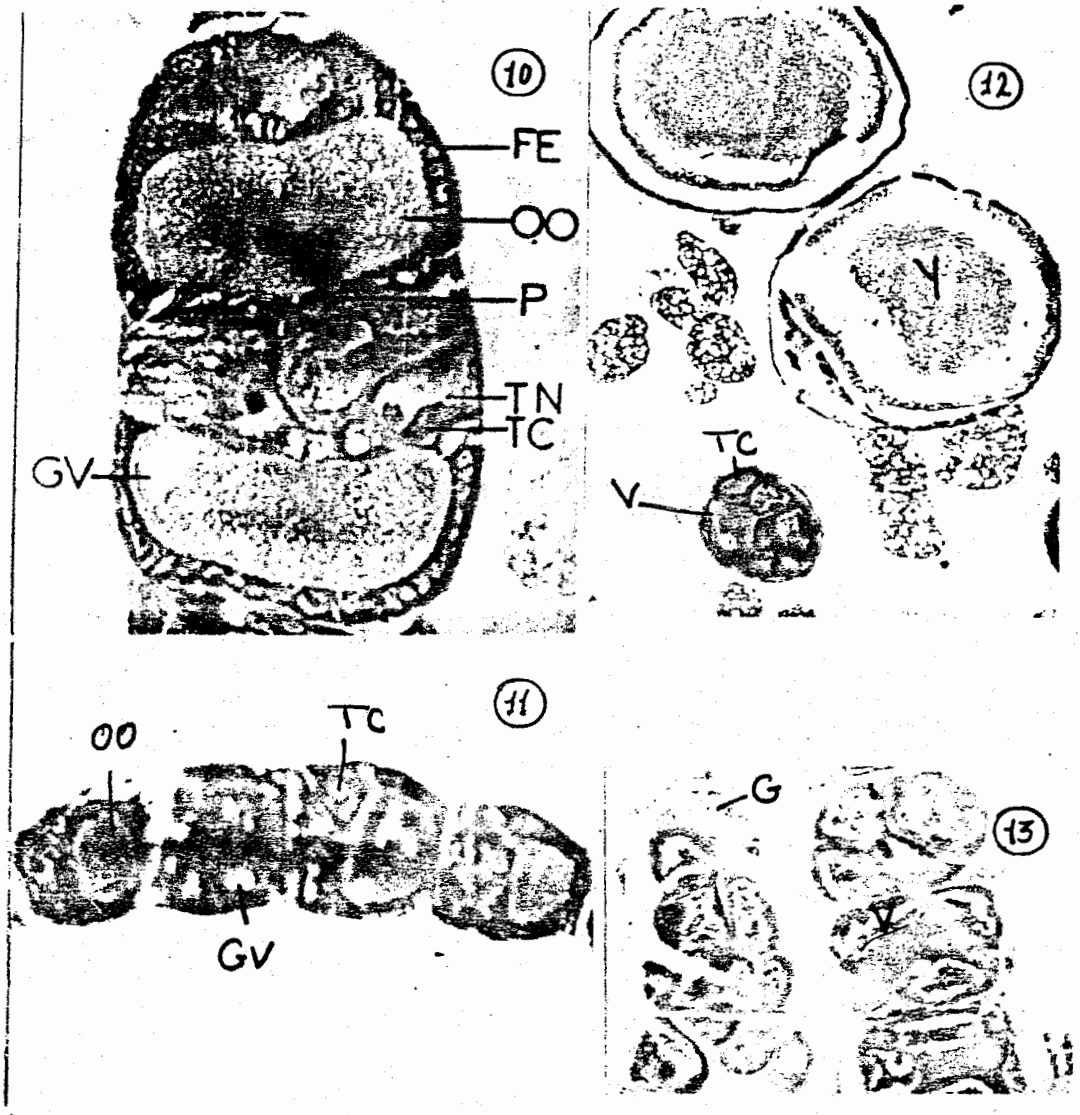
TW (testicular wall), SG (spermatogonla), SC (spermatocyte), SP (spermatid), S(sperm) and V (vacuole).

all testes follicle. They appeared somewhat enlarged. It was difficult to ascertain wheather they were spermatogonia or spermatocytes. In most cases, spermatocytes were pyknotic and had the same appearance as that indicated for five days old pupae treated with 200 Gy. The scattered spermatid bundles were seen among other cell type. In few number of cases a new spermatids were distinguishable. The sperm bundles were seen in a broken state, appeared thick, curved and rod-like in shape. In some testes follicle spermatogonia cells were absent (Fig. 9).

Normal ovaries:

After emergence the female of Spodoptera littoralis has a well developed ovaries (polytrophic type) in which each oocyte has nurse cells enclosed within its follicle.

The wall of the ovariole is formed of a single layer of syncytial epithelial sheath. Inside the ovariole, in the germarium, the follicular epithelial cells can not be easily differentiated from the oogonia. Trophocytes (nurse cell) has a very large nucleus with chromatin material that completely fills the nucleus. The ooplasm and the nurse cells surrounded by the follicular epithelium represent the oocyte. The size of oocyte varies according to the phase of development. The oocyte nucleus (germinal vesicle) is located in the ooplasm. Ova (egg) has a follicular epithelial layer (egg shell) and acquire a yolk (Fig. 10).



Sections in ovary of S. littoralis:
10) Normal ovary of adult S. littoralis.
11) Ovary of adult emerged from irradiated 1-day-old pupae with 50 Gy.
12) Ovary of adult emerged from irradiated 1-day-old pupae with 100 Gy.
13) Ovary of adult emerged from irradiated 3-day-old pupae with 100 Gy.

T(trophocyte),TN(trophocyte nucleus),TC(trophocyte cytoplasm),OO(ooplasm),GV(ooplasm nucleus or germinal vesicle),P(Pore),FE(follicular epithelium), V (vacuole),G(germarium) and OS(outer sheeth).

Treated ovaries:

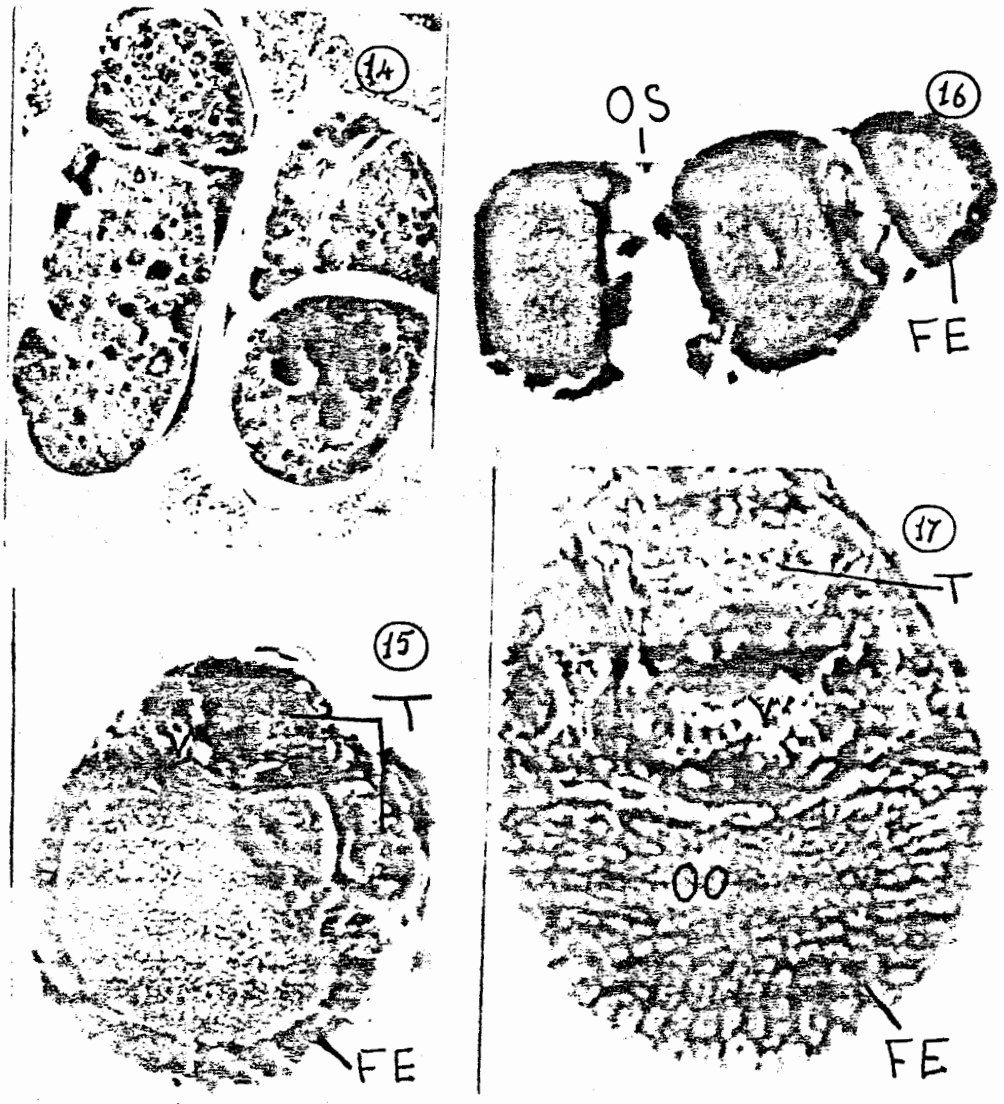
In the ovaries of the adults emerging from one day old irradiated pupae treated with 50 Gy, a little changes were noticed at this low dose. Oogonia, oocytes, and nurse cells appeared more or less normal (Fig. 11).

The ovaries of the adults emerging from one day irradiated pupae treated with 100 Gy, showed more severe changes. These changes include, destruction of the outer sheath enveloping each ovariole and degeneration of young oocytes. The oocytes were hardly seen indicating that their formation had been interrupted. In addition the ooplasm showed evidence of vacuolation. Most oocytes and nurse cells appeared of abnormal shape and were seen clumped together (Fig. 12).

The one day old pupae treated with 150 Gy, failed to emerge adults.

The ovaries of female adults emerged from irradiated three days old pupae with 100 Gy showed apparent destruction of germarium. The process of oocyte formation by oogonia was masked by the large number of similar trophocytes packed into the germarium. Some ova exhibited disorganized cellular arrangement. The yolk of some ova showed evidence of beginning vacuolation. In these cases, the nurse cell nuclei were seen clumped together (Fig. 13).

Histological changes after irradiation by dose 200 Gy



Sections in ovary of S. littoralis:

14) Ovary of adult emerged from irradiated 3-day-old pupae with 200 Gy.
15) Ovary of adult emerged from irradiated 5-day-old pupae with 100 Gy.
16) Ovary of adult emerged from irradiated 5-day-old pupae with 200 Gy.
17) Ovary of adult emerged from irradiated 5-day-old pupae with 300 Gy.

T(trophocyte),TN(trophocyte nucleus),TC(trophocyte cytoplasm),OO(ooplasm),GV(ooplasm nucleus or germinal vesicle),P(Pore),FE(follicular epithelium), V (vacuole),G(germarium) and OS(outer sheeth).

was much more severe and the females were sterile at this level of treatment. Moreover, the oocytes, presented a various degree of alteration reaching to considerable shrinkage of the whole cells. Gross changes were also seen in the nurse cells. Also we can observe deformation of ovariole, with irregular layer of epithelial cells that surround the ovariole. The other germ elements (oogonia, oocytes and nurse cells) were hardly seen. In some cases the cells at the terminal part of the pointed end of the germarium were seen to be sparsely distributed and separated from each other. The compact mass of oogonia which is a characteristic feature of the distal zone of normal ovariole was no longer identifiable (Fig. 14).

Adult females failed to emerge from irradiated three days old pupae of S. littoralis at 300 Gy.

Little change was observed in the ovaries of newly emerged adults from five days old pupae irradiated with 100 Gy. The ovariole wall consists of an outer thin membrane, which looks normal, but has irregular shape. Sometimes separation of the follicular cells from the developing oocyte and ooplasm was observed. Trophocytes and the youngest oocytes appeared distorted. Some oocytes were destructed and nurse cells contained vacuoles (Fig. 15). At 200 Gy irradiated five days old pupae, emerged adults has a more pronounced change than those treated with 100 Gy.

Young oocyte looks normal with vacuoles around the nurse cell nuclei, and there was a decrease of the number of oogonia. Vitellarium sheath around ovary was destructed. Developed egg were still visible in the treated adults, but their shapes were abnormal, and vacuolization of yolk was observed (Fig. 16). The damage occurred in ovarioles of adults emerged from irradiated five days old pupae with 300 Gy was extremely more pronounced than that occurred at doses 100 and 200 Gy. The cytological changes include the disorganization and thickening of the trophocytes and oocytes (Fig. 17).

In all cases atrophy of the ovarioles was obvious. Egg follicles were abnormal in shape and the nurse cell nuclei were clumped together.

DISCUSSION

Our present study showed that in males a dose of 100 Gy would be inadequate for preventing all gonial cell activity. The effects were more pronounced by increasing the dose of irradiation. In females, the egg cells show a different sensitivity according to its degree of development. The young oocytes and oogonia were more sensitive than the oldest one. A dose of 100 Gy was sufficient to cause severe damage to the ovaries. Sperms

and ova were not greatly affected by 50 Gy, but were severely affected at 150 Gy in adult males, 100 Gy in adult females which emerged from irradiated one day old pupae. Transitional cells were damaged at high doses.

We could also verify that the sterilizing dose for adults was 200 Gy for both male and female. However, females of S. littoralis were more sensitive compared with males.

Our present observations are in agreement with that reported by Baccetti and De Dominicis (1964) on Dacus oleae. They showed that, 4 and 5 Krad gamma irradiation applied to pupae caused sterility for adult males. On other hand sterility in females occurred at a dose of 2 Krad.

Offori (1970) on Stomoxys calcitrans, showed that irradiation with 2 Krad did not completely arrest the development of the first egg follicle while 3 Krad resulted in complete atrophy of the ovarioles. Also he indicated that a dose of 5 Krad is sufficient to arrest other process of spermatogenesis and this inpart complete sterility to the male of treated in late pupae or young adults. On the other hand, 4 Krad have no significant effect.

Brower (1973) on Tenebrio molitor, stated that 25 Krad shoved much more severe histological changes in testes and males were sterile at this level of treatment. Whereas sterility was complete in females at 5 Krad.

Ashref and Brower (1974) on Tenebrio molitor, stated that, the severe destruction occur at 25 Krad for adult testes and ovaries and cause strility.

Hodges (1983) on Dermestes frischii, reported that there was a considerable variation in the radiation sensitivity of the various germ cells.

Srivastava et al (1985-1986) on Dysdercus koenigii, mentioned that the testes were completely destroyed and rendered non functioning. They also stated that ovaries showed a pronounced pathological changes including cellular degeneration.

On conclusion we could say that, irradiation caused pronounced damage to both adult ovaries and testes of Spodoptera littoralis emerged from treated pupae. The damage caused by irradiation, to the ovarian and testicular structures was qualitatively similar but varied in intensity. These changes were more obvious at higher dose of irradiation.

Irradiation with doses 200 and 300 Gy, not only delayed ovarian growth but also produced deformation in the shapes of developing follicles and nurse cell nuclei. On the other hand, these doses were found to be sufficient to arrest the process of spermatogenesis which cause complete sterility to the treated males.

REFERENCES

Ashref, M. and Brower, J.H. (1974):
Histological studies of irradiation effects on the gonads of Tenebrio molitor. J. georgia. Ent. Soc. Vg N4, 229-235.

Baccetti, B. and De Dominics, R. (1964):
The effects of gamma radiation on the ovaries of Dacus oleae.
Proceedings of Asymposium, Athens, 22-26 April 1963. Jointly organized by the TAEA and FAO.

Brower, J.H. (1973):
Sensitivity of Tenebrio molitor and T. obscusus to gamma irradiation. J. Eco. Ent. V66: 1175-9

El-Halfawy, N.A. (1983):
Effect of certain radioactive compounds on the reproduction and reproductive system of the cotton leaf worm Spodoptera littoralis.
M.Sc. thesis, Faculty of Science, Ain Shams Unvi.

Hodges, R.J. (1983):
Morphology of the X-irradiated testes of Dermestes frischii Kugelann (Coleop. Dermestidae) J. Inver. Reprod. V6 N1, 35-63.

Offori, E. (1970):
Cytology of gamma-irradiated gonads of Stomoxys calcitrans. Ann. Ent. Soc. Amer., V63 N3, 706-12.

Rosada, J. (1988):
Effects of gamma radiation on testes and on the reproductive ability of the Mediterranean flour moth, Anagasta kuehniella (Lep: Phycitidae) Vienna (Austria): IAEA-ISBN. 369-380.

Srivastava, K.P.; Deshpande, D.J. and Katiyar, R.L. (1985):
Histopathological effect of X-irradiation on the testes of red cotton bug Dysdercus koenigii Fabr. J. Georgia. Ent. V10 N2, 145-149.

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X-irradiation induced histochemical changes in the ovaries of Dysdercus koenigii Fabr. J. Georgia. Ent. V10 N1, 29-33.

التأثيرات النسيجية للتشعيع على مناسل فراشات دودة ورق القطن

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درس تأثير أشعة جاما على مناسل فراشات دودة ورق القطن الناتجة من عذارى معاملة بجرعات مختلفة فى أعمار مختلفة ٠ أحدث التشعيع تأثيرا ضارا وواضحا على كل من الخصية والمبيض ٠ وكان الضرر أكثر وضوحا بزيادة جرعة الاشعاع ٠ ووجد أن الجرعة العالية كان لها تأثيرا أكبر فى احداث عقم خصوصا فى الفراشات الناتجة من عذارى معاملة عمر ٥ أيام وبجرعة مقدارها ٢٠٠ جراى ٠ ومما يستحق الذكر أن الخلايا البالغة كانت أكثر مقاومة من الخلايا المتغيرة ٠